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Scientific Opinion on a request from the European Commission related to the prolongation of prohibition of the placing on the market of genetically modified oilseed rape events Ms8, Rf3 and Ms8 x Rf3 for import, processing and feed uses in Austria

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Abstract: Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) evaluated the documentation provided by Austria to support the prolongation of the safeguard clause measure prohibiting the placing on the market of the genetically modified oilseed rape events Ms8, Rf3 and Ms8 x Rf3 for import, processing and feed uses in Austria. The EFSA GMO Panel assessed whether the submitted documentation comprised new scientific information that would change or invalidate the conclusions of its previous risk assessments on oilseed rape Ms8, Rf3 and Ms8 x Rf3. The EFSA GMO Panel also considered the relevance of the concerns raised by Austria in the light of the most recent data published in the scientific literature. The authorised uses of oilseed rape Ms8, Rf3 and Ms8 x Rf3 exclude cultivation, but data on gene flow, persistence and invasiveness derived from cultivation were considered as a worst case, representing conditions where exposure and potential impact are expected to be the highest, to assess possible environmental impacts resulting from seed import spills. In the documentation provided by Austria and in the scientific literature, the EFSA GMO Panel could not identify new scientific evidence that indicates that the import and processing of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for feed uses in the EU pose a significant and imminent risk to the environment. In conclusion, the EFSA GMO Panel considers that, based on the documentation supplied by Austria and a review of recent scientific literature, there is no specific scientific evidence in terms of risk to the environment that would support the notification of a safeguard clause measure under Article 23 of Directive 2001/18/EC nor its prolongation, and that would invalidate its previous risk assessments of oilseed rape Ms8, Rf3 and Ms8 x Rf3.

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SCIENTIFIC OPINION

Scientific Opinion on a request from the European Commission related to the prolongation of prohibition of the placing on the market of genetically modified oilseed rape events Ms8, Rf3 and Ms8 x Rf3 for import, processing and feed uses in Austria¹

EFSA Panel on Genetically Modified Organisms (GMO)^{2,3}

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ABSTRACT

Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) evaluated the documentation provided by Austria to support the prolongation of the safeguard clause measure prohibiting the placing on the market of the genetically modified oilseed rape events Ms8, Rf3 and Ms8 x Rf3 for import, processing and feed uses in Austria. The EFSA GMO Panel assessed whether the submitted documentation comprised new scientific information that would change or invalidate the conclusions of its previous risk assessments on oilseed rape Ms8, Rf3 and Ms8 x Rf3. The EFSA GMO Panel also considered the relevance of the concerns raised by Austria in the light of the most recent data published in the scientific literature. The authorised uses of oilseed rape Ms8, Rf3 and Ms8 x Rf3 exclude cultivation, but data on gene flow, persistence and invasiveness derived from cultivation were considered as a worst case, representing conditions where exposure and potential impact are expected to be the highest, to assess possible environmental impacts resulting from seed import spills. In the documentation provided by Austria and in the scientific literature, the EFSA GMO Panel could not identify new scientific evidence that indicates that the import and processing of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for feed uses in the EU pose a significant and imminent risk to the environment. In conclusion, the EFSA GMO Panel considers that, based on the documentation supplied by Austria and a review of recent scientific literature, there is no specific scientific evidence in terms of risk to the environment that would support the notification of a safeguard clause measure under Article 23 of Directive 2001/18/EC nor its prolongation, and that would invalidate its previous risk assessments of oilseed rape Ms8, Rf3 and Ms8 x Rf3.

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KEY WORDS

GMO, oilseed rape (*Brassica napus*), Ms8 x Rf3, Austria, safeguard clause measure, environment, Directive 2001/18/EC

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SUMMARY

Following a request from the European Commission, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) evaluated the documentation provided by Austria to support the prolongation of the safeguard clause measure prohibiting the placing on the market of the genetically modified oilseed rape events Ms8, Rf3 and Ms8 x Rf3 for import, processing and feed uses in Austria according to Article 23 of Directive 2001/18/EC.

The EFSA GMO Panel assessed whether the documentation submitted by Austria comprised new scientific information that would change or invalidate the conclusions of its previous risk assessments on oilseed rape Ms8, Rf3 and Ms8 x Rf3. The EFSA GMO Panel also considered the relevance of the concerns raised by Austria in the light of the most recent data published in the scientific literature.

During its evaluation of the supporting documentation, the EFSA GMO Panel observed that the scientific rationale provided by Austria to justify the prolongation of its safeguard clause measure includes data derived from cultivation. The authorised uses of oilseed rape Ms8, Rf3 and Ms8 x Rf3 exclude cultivation, and cover import and processing for food and feed uses only. The EFSA GMO Panel considered the data compiled by Austria and other data on gene flow, persistence and invasiveness derived from cultivation. These data were considered as a worst case, representing conditions where exposure and potential impact are expected to be the highest, to assess possible environmental impacts resulting from seed import spills. However, the EFSA GMO Panel notes that data on gene flow, persistence and invasiveness derived from cultivation represent worst-case conditions that do not reflect those associated with the levels of environmental exposure related to the import, distribution and processing of oilseed rape Ms8, Rf3 and Ms8 x Rf3.

The EFSA GMO Panel also noted that some publications referred to by Austria were already part of the dataset submitted by Austria to support its 2008 safeguard clause measure. These publications were addressed previously by the EFSA GMO Panel in its 2009 Scientific Opinion on the safeguard clause measure invoked by Austria on oilseed rape Ms8, Rf3 and Ms8 x Rf3.

The EFSA GMO Panel could not identify new scientific evidence in the documentation provided by Austria and in the scientific literature that indicated that the import and processing of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for feed uses in the EU pose a significant and imminent risk to the environment.

In conclusion, the EFSA GMO Panel considers that, based on the documentation supplied by Austria, and a review of recent scientific literature, there is no specific scientific evidence in terms of risk to the environment that would support the notification of a safeguard clause measure under Article 23 of Directive 2001/18/EC nor its prolongation, and that would invalidate its previous risk assessments of oilseed rape Ms8, Rf3 and Ms8 x Rf3.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The import and processing of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for some food uses (oil and additives), feed uses and other uses than food and feed are authorised in the European Union⁴. These GMOs are currently under renewal of their authorisation for the food uses and some feed uses (feed materials and additives).

In July 2008, Austria notified to the European Commission a national safeguard measure prohibiting the placing on the market of oilseed rape Ms8, Rf3 and Ms8 x Rf3 in Austria. Austria provided a scientific argumentation in support of its decision.

In June 2009, EFSA issued a scientific opinion concluding that there was no scientific evidence justifying the invocation of a safeguard clause under Article 23 of Directive 2001/18/EC for the marketing of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for its intended uses in Austria.

In July 2011, Austria notified to the European Commission its Ordinance BGBl. II No 305/2010 of 28/09/2010 prolonging the implementation of the national safeguard measure prohibiting the placing on the market of oilseed rape Ms8, Rf3 and Ms8 x Rf3 in Austria. Austria also provided new scientific elements in support of its decision.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA was requested in accordance with Article 29 of Regulation (EC) No 178/2002 to assess the new scientific information submitted by the Austrian Authorities justifying prolongation of their national safeguard measure concerning GM oilseed rapes Ms8, Rf3 and Ms8 x Rf3 and to identify whether these new scientific elements might lead the GMO Panel to reconsider its related favourable opinions on GM oilseed rapes Ms8, Rf3 and Ms8 x Rf3 from 2005 and 2009.

⁴ See EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

ASSESSMENT

1. INTRODUCTION

Austria previously invoked national safeguard clause measures to provisionally prohibit the marketing of specific oilseed rape events GT73 (Verordnung, 2006) and Ms8, Rf3 and Ms8 x Rf3 (Verordnung, 2008) on its territory.

The EFSA GMO Panel previously evaluated these two Austrian national bans, and concluded that, in terms of risk to the environment, no new scientific evidence had been presented (EFSA, 2009a,b) that would invalidate the previous risk evaluations of genetically modified herbicide tolerant (GMHT) oilseed rape (EFSA, 2004a, 2005, 2008). EFSA reiterated its opinion that unintended environmental effects due to the accidental spillage of GMHT oilseed rape seed will be no different from that of conventional oilseed rape (see also EFSA, 2004b, 2006b).

In July 2011, Austria provided new scientific elements to prolong the implementation of the national safeguard measure prohibiting the placing on the market of oilseed rape Ms8, Rf3 and Ms8 x Rf3 in Austria.

The EFSA GMO Panel examined the documentation submitted in 2011 by Austria. In line with the terms of reference set by the European Commission, the EFSA GMO Panel assessed whether the submitted documentation comprises new scientific information that would change or invalidate the conclusions of its previous risk assessments on oilseed rape Ms8, Rf3 and Ms8 x Rf3.

In addition, the EFSA GMO Panel looked for evidence for GMO-specific risks taking into consideration its Scientific Opinion delivering guidance on the environmental risk assessment of GM plants (EFSA, 2006a, 2010), as well as any related risk assessments carried out previously (EFSA, 2005, 2009b,c). The EFSA GMO Panel also considered the relevance of the Austrian concerns in the light of the most recent scientific data published in the scientific literature. Since the risk assessment strategy for GM plants seeks to compare the GM plant with a conventional counterpart (EFSA, 2006a, 2010, 2011a,b), non-GM oilseed rape was taken as a comparator. Relevant data on feral plants derived from cultivation (as distinct from import) were considered as a worst case, representing conditions where exposure and potential environmental impacts are expected to be the highest. These data were used to assess the role of feral GMHT oilseed rape resulting from seed import spills (see also Devos et al., 2012). In the assessment of oilseed rape Ms8, Rf3 and Ms8 x Rf3, the EFSA GMO Panel also considered relevant information on other herbicide tolerant oilseed rape events, in particular oilseed rape GT73 and T45.

2. CONCERNS RAISED BY AUSTRIA

The EFSA GMO Panel interprets the documentation provided by Austria as raising the following issues:

- imports of viable oilseed rape Ms8 x Rf3 seeds in Austria are substantial and can be transported inland (see section 4 of this Scientific Opinion);
- imported oilseed rape Ms8 x Rf3 seeds will escape through spillage (section 5);
- spilled oilseed rape Ms8 x Rf3 seeds will persist outside agricultural fields as feral plants (section 6);
- feral oilseed rape Ms8 x Rf3 plants may extend the potential for gene flow by acting as stepping stones and by forming populations that accumulate transgenes, thereby contributing to admixtures with commercially grown oilseed rape varieties (section 7);

- feral oilseed rape Ms8 x Rf3 will mediate transgene movement towards sexually compatible plants in the landscape (section 8);
- herbicide tolerance traits may cause a change in fitness, leading to invasion of (semi-)natural habitats, and a colonisation of agricultural fields (section 9);
- feral oilseed rape Ms8 x Rf3 may cause or exacerbate herbicide management problems of roadside habitats due to the unintended stacking of herbicide tolerance traits (section 10).

3. BACKGROUND INFORMATION ON VERTICAL GENE FLOW IN OILSEED RAPE

Oilseed rape is an open pollinating crop plant capable of cross-pollinating with other Brassica crops (Eastham and Sweet, 2004) and some wild relatives (Devos et al., 2009a). It produces large amounts of small seeds which can survive and persist for many years in soil (Lutman et al., 2004, 2005, 2008; Begg et al., 2006; Messéan et al., 2007; D'Hertefeldt et al., 2008; Gruber et al., 2008; Andersen et al., 2010; Beckie and Warwick, 2010; Munier et al., 2012) and which tend to be widely dispersed during farm and transport operations (Price et al., 1996; Zwaenepoel et al., 2006; von der Lippe and Kowarik, 2007b; Pivard et al., 2008a,b; Bailleul et al., 2012). Seed dispersal results in oilseed rape being a major weed (volunteer) in crop rotations and the occurrence of feral plants outside cultivated areas, often in ruderal – non-cropped, disturbed – habitats, where they can survive and reproduce successfully without management (Gressel, 2005; Bagavathiannan and Van Acker, 2008). In areas where oilseed rape is cultivated, feral oilseed rape typically originates either from the spillage of seed during its transport to and from fields and to processing plants, the redistribution of seed by field equipment and grain trailers (Bailleul et al., 2012), or the dispersal of seed, for example by birds and mammals (von der Lippe and Kowarik, 2007a,b; Wichmann et al., 2009). Transport of seeds following both cultivation and importation has resulted in dispersal of seeds into a range of environments. Volunteer populations in agricultural fields arise mostly from seeds lost through the shattering of the seed-bearing pods before and during harvest. At seed maturity the pods become fragile and easily split open, resulting in losses that can reach up to 10% of the seed yield (Thomas et al., 1991; Price et al., 1996; Morgan et al., 1998; Hobson and Bruce, 2002; Gulden et al., 2003a).

4. IMPORTS OF VIABLE OILSEED RAPE SEEDS TO THE EU

4.1. Concern raised by Austria

Austria raised the concern that imports of viable oilseed rape Ms8 x Rf3 seeds in Austria are substantial and can be transported inland.

4.2. Assessment by EFSA GMO Panel

4.2.1. Import characteristics

Import of viable seed for use in the oilseed rape crushing industry from overseas countries where GMHT oilseed rape is currently grown commercially (e.g., Australia, Canada and the USA) is entirely in bulk (i.e., large containers rather than handy-sized bags) and by boat. While most (GMHT) oilseed rape seed imported from overseas is crushed in or near the ports of entry in the EU, a fraction of it can be transported inland to small independent processing (crushing) facilities by boat, truck or rail (Tamis and de Jong, 2010; Schoenenberger and D'Andrea, 2012). Because it is uneconomical to transport imported viable seed inland for processing in landlocked processing facilities, it is mainly transported by boat to river-located ports (EFSA, 2004a), where it is usually unloaded by pneumatic discharge, by crane in sealed crates, or by a screw conveyor in a sealed tube. The unloaded material is deposited on a conveyor belt that takes it to a quayside storage silo from where it is dispatched by truck to a storage site at the processing facility (Tamis and de Jong, 2010). Evidence indicates that viable oilseed rape is mostly processed on-site and has little travelling distance between the points of entry and processing (Tamis and de Jong, 2010). Smaller independent crushing facilities located inland away from rivers tend to supply themselves from domestic production (EFSA, 2004a), as these facilities market the oil they produce on the basis of locality and provenance. According to Tamis and de Jong (2010), the

only route by which small amounts of imported (GMHT) oilseed rape seeds may escape into the wider environment is during the distribution and marketing of seed used for the production of food for birds.

In 2010, 9,118,000 tons of viable oilseed rape seeds were imported to EU countries⁵. Belgium, France, Germany and the Netherlands were the main importers of viable oilseed rape seeds, accounting for 76% of the total volumes imported in the EU in 2010.

Approximately 21% of imports of viable oilseed rape seeds came from outside the EU, including Ukraine and overseas countries (mainly Australia and New Zealand, but also Argentina, Canada and the USA) in 2010. The estimation of the approximate share of GMHT oilseed rape cultivations in the overseas GMHT oilseed growing countries gives an indication of the amount of transgenic oilseed rape that could possibly be imported into the EU. Even though the import figures vary annually with the changing domestic production of oilseed rape within EU Member States and market demands, the main importers of viable oilseed rape seeds from outside the Community are Belgium, France, Germany, Portugal, and the Netherlands. Preliminary data indicate that imports of oilseed rape seeds (mainly from Canada and Australia) are likely to increase compared to 2009-2011 due to low domestic EU-27 production.

STATISTIK AUSTRIA⁶ reported that Austria imported 274,705 tons of viable oilseed rape seeds in 2010-2011, and Fediol indicated that 304,000 tons has been imported in 2010, of which 5,000 tons came from outside the EU.

4.2.2. Conclusion

The import volume of viable oilseed rape seeds from outside the EU was 5,000 tons in Austria in 2010. Most of this was imported in bulk containers for processing in the main ports on the river Danube and connecting waterways. Little, if any, imported viable seed is currently transported overland away from these main ports and processing facilities.

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that *“the amounts of viable oilseed rape seeds imported in the EU are limited with most seeds being imported by boat and crushed in or near the ports of entry”* (EFSA, 2009a).

5. OCCURRENCE OF FERAL GMHT OILSEED RAPE

5.1. Concern raised by Austria

Austria raised the concern that imported oilseed rape Ms8 x Rf3 seeds will escape through spillage.

5.2. Assessment by EFSA GMO Panel

5.2.1. Monitoring surveys

Several monitoring surveys, assessing the presence of transgenes in feral oilseed rape populations, have been conducted (Appendix A).

5.2.1.1. Cultivation scenario

In regions where GMHT oilseed rape is widely grown such as western Canada and the USA, monitoring surveys confirmed the widespread occurrence of feral GMHT oilseed rape plants along field margins of agricultural fields, as well as along transportation routes (such as road verges and railway lines). In the study of Yoshimura et al. (2006), approximately 2/3 of the feral plants sampled were transgenic, whereas all feral plants sampled by Knispel et al. (2008) exhibited the presence of the

⁵ http://www.fediol.be/data/Stat_seeds_2010.pdf

⁶ http://www.statistik.at/web_de/static/versorgungsbilanz_fuer_oelstaaten_200506_-_201011_022320.pdf

glyphosate (GLY) or glufosinate-ammonium (GLU) tolerance traits (or both). In North Dakota (USA), 80% (231/288) of the sampled feral oilseed rape plants expressed at least one herbicide trait (CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and phosphinothricin-N-acetyltransferase (PAT)): 41% (117/288) of the plants were positive for only CP4 EPSPS and 39% (112/288) were positive for the PAT; and 0.7% (2/288) expressed both herbicide tolerance traits (Schafer et al., 2010, 2011). The presence of feral GMHT oilseed rape plants was also detected at the port of Vancouver on the west coast of Canada, where most GMHT oilseed rape seed for export is transported by rail (Yoshimura et al., 2006). These data indicate that feral GMHT oilseed rape will be present along roadsides and other ruderal habitats in areas where GMHT oilseed rape is commercially grown or at points from where it is exported. The frequency of transgenes corresponds approximately to the proportion of oilseed rape grown or in transit that is transgenic (Yoshimura et al., 2006; Knispel et al., 2008). The available data suggests that the frequency of occurrence of feral GMHT plants will be similar to that of conventional feral plants under similar environmental and exposure conditions. Differences in populations are only likely to occur where the associated herbicides (in this case GLU) are applied to the GMHT feral plants (section 9).

5.2.1.2. Import scenario

In regions where GMHT oilseed rape is currently not grown commercially, surveys performed in and around major ports and along roads leading from these ports to inland processing facilities in Japan, revealed that feral oilseed rape plants can express/contain the GLY or GLU tolerance trait, and to a lesser extent both traits (Saji et al., 2005; Aono et al., 2006; Kawata et al., 2009; Nishizawa et al., 2009). The share of feral plants that was transgenic varied substantially across years and sampling sites, ranging from 0.2% to 100% (Kawata et al., 2009; Nishizawa et al., 2009). Aono et al. (2006) also reported the presence of *barnase* and *barstar* genes in the progeny of some of the sampled oilseed rape plants. Since no GM oilseed rape has been grown for marketing purposes in Japan (Nishizawa et al., 2010), transgene presence was attributed to the accidental loss and spillage of imported viable GMHT oilseed rape seed.

In the EU, Mbongolo Mbella et al. (2010) analysed 20 samples (each containing leaf material from five adjacent (clustered) oilseed rape plants) collected in Belgian port areas, and did not detect the presence of transgenes in these samples. Schoenenberger and D'Andrea (2012) surveyed 31 Swiss railway stations. From the 1,242 sampled oilseed rape plants, 21 plants were positive for CP4 EPSPS (1.7%).

Extensive monitoring surveys, assessing transgene presence in feral populations, as those performed in Japan have not been reported for EU countries. Therefore, caution is recommended when extrapolating the reported instances of feral GMHT oilseed rape in and around major ports and along roads leading from these ports to inland processing facilities in Japan to European environments. Moreover, among EU countries, the origin and volumes of imported (GMHT) oilseed rape seeds, the potential use of inland processing facilities, and the receiving environments into which seed spills may occur (e.g., vegetation density and composition, type and timing of road verge management) may differ from those observed in Japan. For instance, Kawata et al. (2009) indicated that Japan imports over 2,000,000 tons of viable oilseed rape seeds each year, mostly from Canada. Further, whether spilled seed will germinate, establish seedlings and lead to feral oilseed rape plants that flower and set seed is largely dependent on the characteristics of the receiving environment.

5.2.2. Conclusion

The above data indicate that seed spillage of GMHT oilseed rape will occur wherever it is transported or cultivated, and that feral plants are likely to be present along transportation routes in all countries cultivating and/or receiving imports of viable seeds of GMHT oilseed rape. Seed spillage is a random event and therefore the levels of seed immigration can vary substantially. Further, whether spilled seed will germinate, establish seedlings and lead to feral oilseed rape plants that flower and set seed is largely dependent on the characteristics of the receiving environment. As the import volumes of viable oilseed rape seeds from outside the EU are minimal (section 4.2.2), the occurrence of feral GMHT

oilseed rape resulting from seed import spills is likely to be low and mostly confined to port areas. Therefore, the environmental exposure due to GMHT oilseed rape seed imports is anticipated to be low.

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that “*feral oilseed rape plants are likely to occur wherever oilseed rape is cultivated and/or transported and that transgenic oilseed rape is no exception (e.g., Saji et al., 2005; Aono et al., 2006; Yoshimura et al., 2006; Bagavathiannan and Van Acker, 2008; Kawata et al., 2008; Knispel et al., 2008; Nishizawa et al., 2009)*”, but that “*the environmental exposure due to GM oilseed rape grain imports is anticipated to be low, as the amounts of viable oilseed rape seeds imported in the EU are limited with most seeds being imported by boat and crushed in or near the ports of entry*” (EFSA, 2009a).

The EFSA GMO Panel reiterates that it does not consider the occasional occurrence of feral GMHT oilseed rape plants as an environmental hazard in itself, and is primarily concerned with assessing the environmental consequences of this occurrence on biotic interactions and ecosystems (section 9; EFSA, 2006a, 2010).

6. PERSISTENCE AND POPULATION DEMOGRAPHICS OF FERAL OILSEED RAPE

6.1. Concern raised by Austria

Austria raised the concern that spilled oilseed rape Ms8 x Rf3 seeds will persist outside agricultural fields as feral plants.

6.2. Assessment by EFSA GMO Panel

6.2.1. Population characteristics

Feral oilseed rape has been reported in several regions (Appendix B) and occurs in ruderal habitats such as field margins, road verges, paths, ditches, railway lines, building sites, ports, seed handling, storage and processing facilities, and wastelands. A population can be defined as a single plant or group of plants that is spatially separated from another feral population. The size of such populations ranges from single plants to stands of over 1,000 plants with the majority of populations containing 100 plants or less (Squire et al., 2011). Comparisons of five demographic studies of feral oilseed rape in different EU locations (Denmark, Germany (2), France and the UK), constituting over 1,500 ha and 16 site-years of observations, showed that feral populations generally occur at relatively low densities, with a mean around one population per square kilometre, rising to 15 per square kilometre in areas with a high frequency of oilseed rape cultivation such as a study site at Selommès, Loir-et-Cher, France (Lecomte et al., 2007; Messéan et al., 2009; Squire et al., 2011). The spatial variation in feral populations in part reflects differences in frequency of oilseed rape cultivation and abundance of in-field oilseed rape volunteers in the landscape (Knispel and McLachlan, 2009).

6.2.2. Population demography and factors contributing to persistence

Oilseed rape is generally regarded as an opportunistic species, which can take advantage of disturbed sites due to its potential to germinate and capture resources rapidly. In undisturbed natural habitats, oilseed rape lacks the ability to establish stable populations, possibly due to the absence of competition-free germination sites (Crawley et al., 1993, 2001; Warwick et al., 1999; Hails et al., 2006; Sutherland et al., 2006; Damgaard and Kjaer, 2009). Moreover, in controlled sowings into road verges, field margins and wasteland, very few seedlings survived to maturity due to grazing (e.g., by molluscs) and abiotic stress (Charters et al., 1999).

Once established in competition-free germination sites, feral populations decline over a period of years. A 10-year survey (1993-2002), along road verges of a motorway revealed that most quadrats showed transient populations lasting one to four years (Crawley and Brown, 2004). These data and

data from other demographic studies indicate a substantial turnover of populations of feral oilseed rape: only a small percentage of populations occurs at the same location over successive years, but the majority of plants did not survive, resulting in rapidly declining populations (Crawley and Brown, 1995, 2004; Charters et al., 1999; Peltzer et al., 2008; Elling et al., 2009; Knispel and McLachlan, 2009; Nishizawa et al., 2009; Mizuguti et al., 2011; Squire et al., 2011). However, if habitats are disturbed on a regular basis by anthropogenic activities such as mowing, herbicide applications or soil disturbance, or natural occurrences such as flooding, then feral populations can persist for longer periods (Claessen et al., 2005a; Garnier et al., 2006).

The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, to recruitment from seed emerging from the soil seedbank or shed by resident feral adult plants, or to redistribution of feral seed from one location to another. The respective contribution of these input sources is still a matter of discussion.

6.2.2.1. Replenishment with fresh seed spills

Because feral oilseed rape is more prevalent in areas with a high frequency of oilseed rape cultivation (Squire et al., 2011), along high-traffic roadsides (Crawley and Brown, 1995, 2004; Knispel and McLachlan, 2009), and in the proximity to seed handling, storage and processing facilities (Yoshimura et al., 2006; Peltzer et al., 2008), repeated seed immigration from both agricultural fields and transport (as fresh seed spills) has been considered the main source contributing to population persistence, countering high declines or extinction rates at a local scale. Few studies have been able to define the proportion of populations derived from fresh spills, but at the study site of Selommès in France, 15% of feral populations were attributed to immigration through seed transport, potentially including seed imports to the area, as opposed to 35-40% originating from seed from neighbouring fields (Pivard et al., 2008a). In a follow-up study, Bailleul et al. (2012), during a period eight sampling days, collected a total of 7,710 oilseed rape seeds in 85 trap-sites placed in the vicinity of a grain silo where locally produced oilseed rape seed is stored. The authors also reported that 80% of the seeds collected after seed spillage germinated to seedlings under optimal greenhouse conditions. As most EU receiving environments represent less suitable habitats than the greenhouse, the rate of seed germination to seedlings is likely to be much lower under real conditions, but sufficient to contribute to population persistence. This indicates that the rate of successful replenishment will not only depend on the volumes and frequencies of fresh seed spills, but also and mainly on the characteristics of the receiving environments (section 9).

6.2.2.2. Recruitment from seed emerging from the soil seedbank

The dynamics of feral populations at one location also depend on soil seedbanks (Pivard et al., 2008b). Demographic data on feral oilseed rape in different EU locations showed consistently that persistence in the soil seedbank allowed plants to recur after an absence of a year or more, while several populations persisted for two to four years (Squire et al., 2011). For the study site of Selommès, Pivard et al. (2008a) estimated that up to 40% of the observed feral populations persisted mainly through seed emerging from the soil seedbank. There is a large body of evidence from the study of volunteers showing that oilseed rape seed can remain in secondary dormancy for many years in the soil seedbank, and germinate in subsequent years. Under field conditions, the persistence of secondarily dormant seed has been confirmed to be up to five years, but may reach ten years or more (Simard et al., 2002; Gulden et al., 2003b; Lutman et al., 2004, 2005, 2008; Begg et al., 2006; Messéan et al., 2007; Jørgensen et al., 2007; D'Hertefeldt et al., 2008; Gruber et al., 2008; Andersen et al., 2010; Beckie and Warwick, 2010; Munier et al., 2012). Secondary dormancy is complex: it can be induced by a range of factors such as low temperature, soil dryness, and darkness through burial in soil (López-Granados and Lutman, 1998; Squire, 1999; Marshall et al., 2000; Momoh et al., 2002; Gruber et al., 2004, 2010; Gulden et al., 2004a,b), and is genotype-dependent (Gulden et al., 2004a, Gruber et al., 2010; Thöle and Dietz-Pfeilstetter, 2012). Recently, dormant oilseed rape seed has been found in the soil seedbank in non-till systems, indicating that seed can fall dormant on the soil surface, and need not to be buried in the dark (Gruber et al., 2010).

However, evidence on the contribution of seed from the seedbank is not entirely consistent. Biochemical and molecular analyses indicated that feral sites can contain plants with the same varietal profile consistently for at least three years, and can contain varieties last commercially grown three or more years previously (Squire et al., 2011). Since individual varieties of oilseed rape are sown for only a few years before being superseded by new varieties, the existence of markers from previous varieties indicates the possibility they persisted as ferals, provided origins from farm-saved seed or persistent volunteers can be ruled out (SIGMEA, 2010). Biochemical and genetic analyses, in conjunction with farmer surveys, established the persistence of varieties no longer grown or marketed for at least five years in Austria (Pascher et al., 2006) and eight years in France (Pessel et al., 2001). In a continuation of the study by Charters et al. (1999), it was observed that one population contained, over a period of twelve years, a genetic signature of a variety that had been obsolete for at least ten of those years (see also Banks et al., 2010). In contrast, based on a preliminary analyses of soil samples at feral oilseed rape roadside sites in western Canada in the greenhouse, Knispel et al. (2008) indicated that feral oilseed rape roadside soil seedbanks are small (less than five viable seeds per square metre) and lack substantive dormancy. In total, however, the observations from Europe indicate that feral populations have been sufficiently consistent in their presence and abundance to act as a genetic bridge between past and current oilseed rape varieties.

6.2.2.3. Replenishment of the seedbank by resident feral oilseed rape plants

The dynamics of feral populations at one location also depend on local recruitment from seed produced by resident feral plants (Pivard et al., 2008b). Even though observations from demographic studies across Europe showed that seed yield of feral plants is often much smaller than that of the crop due to the less suitable habitat than agricultural fields, seed from mature plants is still likely to replenish the soil seedbank and contribute to population persistence (Squire et al., 2011). One of the few direct estimates in Europe is by Pivard et al. (2008a) who found that local seed input from resident feral oilseed rape is rare, accounting for less than 10% of subsequent feral populations in the study site of Selommes. Other data, relying on the existence of feral plants bearing seed, are mostly circumstantial and indicate that the proportions of feral plants having pods ranged between 30 to 48% in northwest Germany (Elling et al. 2009). These values are two to three times higher than those observed in Selommes, while in western Canada, the seed yield from individual feral plants was comparable to that of the crop (Knispel et al., 2008).

6.2.2.4. Redistribution of feral seed between local populations

The dynamics of feral populations at one location also depend on redistribution of feral seed between local populations (Pivard et al., 2008b). Therefore, the feral seedbank could in principle consist of seed brought into the location from outside and seed from plants reproducing on site (section 6.2.2.3). Seed brought in from outside could be carried by vehicles, road verge mowers, animals, or by the movement of soil for agricultural and building works (Wilkinson et al., 1995; Garnier et al., 2008; Wichmann et al., 2009). Garnier et al. (2008) showed that wind turbulence behind passing vehicles locally contributed to the secondary dispersal of seed: on average, 20% of the seed was estimated to disperse over a few metres, while 80% of the seed remained at the original place. However, there is little evidence of the contribution of such redistributed seed compared to that of seed deposited by plants reproducing on site.

6.2.3. Conclusion

Feral oilseed rape is part of a dynamic metapopulation of plants in which the most numerous are crop plants and volunteers (Simard et al., 2005; Gruber and Claupein, 2007; Messéan et al., 2009; Knispel, 2010; Middelhoff et al., 2011; Squire et al. 2011). The evidence indicates that oilseed rape is capable of establishing self-perpetuating populations outside agricultural areas. While many feral populations observed over multiple years were transient at a local scale (e.g., Crawley and Brown, 1995, 2004; Knispel et al., 2008), this apparent transience is likely counterbalanced at a landscape scale by repeated seed addition and redistribution from various sources. Local declines or extinctions in above-ground feral populations are likely to be temporary and asynchronous at large spatial scales (Charters

et al., 1999; Crawley and Brown, 2004; Peltzer et al., 2008; Knispel and McLachlan, 2009; Nishizawa et al., 2009). On a larger scale in the landscape, feral oilseed rape can thus be considered long-lived with a proportion of the populations founded by repeated fresh seed spills from both agricultural fields and transport, and the remainder resulting from the continuous recruitment of seed from local feral soil seedbanks.

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that “*in regions where oilseed rape is grown and/or where oilseed rape seeds are imported and transported, feral oilseed rape populations are likely to occur in non-natural disturbed ecosystems (such as ports, processing facilities, margins of agricultural fields, roadside verges, railway lines, and wastelands)*”. In addition, the EFSA Panel indicated that “*in most non-agricultural areas, oilseed rape lacks the ability to establish stable populations due to the absence of competition-free gaps (Crawley et al., 1993; Warwick et al., 1999; Hails et al., 2006). Once established, oilseed rape populations often become extinct after 2 to 4 years (Crawley and Brown, 1995; Crawley et al., 2001; Norris and Sweet, 2002). If habitats are disturbed on a regular basis (e.g., by mowing, herbicide application, soil disturbance) and replenished with seed from seed spillage or recruitment from seeds produced by residents or from seeds from the seedbank, then feral oilseed rape populations can persist for longer periods (8-10 years) (Pessel et al., 2001; Pivard et al., 2008a,b)*” (EFSA, 2009a).

7. GENE FLOW FROM FERAL OILSEED RAPE TO OTHER OILSEED RAPE VARIETIES

7.1. Concern raised by Austria

Austria raised the concern that feral oilseed rape Ms8 x Rf3 plants may extend the potential for gene flow by acting as stepping stones and by forming populations that accumulate transgenes, thereby contributing to admixtures with commercially grown oilseed rape varieties.

7.2. Assessment by EFSA GMO Panel

7.2.1. *Feral oilseed rape as the receptor plant – crop-to-feral gene flow*

7.2.1.1. Cultivation scenario

Oilseed rape is an outcrossing species with potential to cross-pollinate other oilseed rape types at varying levels of frequency depending on flowering synchrony, spatial arrangement of plants, presence of pollinator insects and other factors as reviewed by Eastham and Sweet (2004) (see also Hüskén and Dietz-Pfeilstetter, 2007; Beckie and Hall, 2008; Devos et al., 2009a). Few direct measurements to quantify crossings between commercially grown oilseed rape and feral plants have been made so far, but the fact that crossing occurs, and hence genomes of old and new varieties combine, was demonstrated at several localities in the EU (Charters et al., 1999; Bond et al., 2004; Dietz-Pfeilstetter et al., 2006, 2012; Pascher et al., 2006, 2010; Elling et al., 2009). More generally, the potential for cross-fertilisation of feral plants by the crop plant simultaneously in flower over a range of distances has been demonstrated by the use of small groups of male-sterile recipient plants distributed in the landscape (Ramsay et al., 2003; Devaux et al., 2005, 2007, 2008; Chifflet et al., 2011). Using male-sterile plants (which produce no pollen of their own) as recipients tends to overestimate the actual frequency of cross-fertilisation that would occur between the crop plants and pollen-fertile ferals by more than 10-fold (Ramsay et al., 2003; GR Squire, unpublished data), but demonstrates the potential for its occurrence. This approach combined with modelling work confirmed that cross-fertilisation levels usually decline very steeply with distance from one field to an adjacent or nearby field (Hüskén and Dietz-Pfeilstetter, 2007; Beckie and Hall, 2008), but they occur at low frequency over several kilometres (Rieger et al., 2002; Ramsay et al., 2003; Devaux et al., 2005, 2007, 2008; Chifflet et al., 2011). It is expected that crossing of the order of 1 to 10% will occur to feral plants a few metres from a donor, and of 0.1 to 0.01% to ferals that are 100 m away (SIGMEA, 2010). Since feral plants are widespread in some agricultural regions and occur in close proximity to

commercially grown oilseed rape in flower, most feral plants in agricultural landscapes would be exposed to pollen from crops. In the major demographic studies of oilseed rape in Europe, the proximity of feral populations to the nearest flowering field of oilseed rape was measured in four of the study areas: approximately 10% of the ferals were within 10 m; 15% within 100 m (50% at the study side of Selommes) and 80% within 1,000 m (SIGMEA, 2010). This suggests that feral plants, even lasting only one year, can be cross-fertilised by commercially grown oilseed rape and have the potential to accumulate transgenes in areas where GMHT oilseed rape is grown.

In western Canada where GMHT oilseed rape is widely grown, pollen-mediated gene flow has resulted in the unintended stacking of herbicide tolerance traits in both volunteer (Hall et al., 2000; Beckie et al., 2003) and feral plants (Knispel et al., 2008). Even though Yoshimura et al. (2006) failed to detect feral plants with both herbicide tolerance traits in western Canada, the authors argued that such plants would likely have been detected with more intensive sampling. Also, in North Dakota (USA), two instances of unintentionally stacked traits have been reported recently (Schafer et al., 2010, 2011). It is likely that adjacent plants within feral populations may further contribute to the spread and stacking of herbicide tolerance traits, especially where feral plants with different herbicide tolerance traits occur together (Knispel et al., 2008), as cross-fertilisation rates increase with increasing proximity of oilseed rape plants (Funk et al., 2006).

7.2.1.2. Import scenario

Due to the relative scarcity of feral plants, the most plausible source for unintended stacking under an import scenario is through the cross-fertilisation between plants having different herbicide tolerance traits in the country of origin, and the spillage of this unintentionally stacked HT oilseed rape seed subsequently imported in the EU. In Japan, where GMHT oilseed rape is not grown commercially, but viable oilseed rape seed is imported, a portion of the progeny of two feral plants has been shown to contain both the GLY and GLU tolerance traits (Aono et al., 2006). The authors could not conclusively determine whether the double HT progeny resulted from cross-fertilisations between adjacent plants with different herbicide tolerance traits in Japan, or from the import of double HT seed unintentionally stacked in Canada. However, import seems the most reasonable explanation, as the unintended stacking of herbicide tolerance traits in certified seed (Friesen et al., 2003; Demeke et al., 2006) was reported in Canada, whereas other extensive surveys of feral plants conducted in Japan failed to detect feral plants with multiple transgenes (Saji et al., 2005; Kawata et al., 2009; Nishizawa et al., 2009).

7.2.1.3. Conclusion

Based on the available scientific literature, the EFSA GMO Panel concludes that feral plants can be cross-fertilised by commercially grown oilseed rape and have the potential to accumulate transgenes in areas where GMHT oilseed rape is grown. However, while theoretically possible, the most plausible source for unintended stacking under an import scenario is through the cross-fertilisation between plants having different herbicide tolerance traits in the country of origin, and the spillage of this unintentionally stacked HT oilseed rape seed subsequently imported in the EU.

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that “*feral oilseed rape populations may serve as a reservoir that could hold and return (trans)genes to cultivated populations of oilseed rape in a different place and time (Saji et al., 2005; Aono et al., 2006; Pascher et al., 2006; Yoshimura et al., 2006; Knispel et al., 2008; Nishizawa et al., 2009)*” (EFSA, 2009a).

The EFSA GMO Panel reiterates that it does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on biotic interactions and ecosystems (section 9; EFSA, 2006a, 2010).

7.2.2. *Feral oilseed rape as the donor plant – feral-to-crop gene flow*

7.2.2.1. Cultivation scenario

Feral (GMHT) oilseed rape plants could pollinate crop plants of non-GM oilseed rape if feral populations are immediately adjacent to field crops (Garnier and Lecomte, 2006). The contribution of feral plants to pollen flow into agricultural fields has been argued to be extremely small compared to that from the crop plants and volunteers, simply because of the far smaller number of feral plants (Ramsay et al., 2003; Gruber and Claupein, 2007; Messéan et al., 2009; Middelhoff et al., 2011; Squire et al., 2011). The main channel by which herbicide tolerance traits persist over time in fields would be through volunteers. In the major demographic studies of oilseed rape in Europe, the highest percentage of flowering feral plants was around 0.002% (two flowering feral plants for 100,000 crop plants) and the percentage of seed on feral plants was in all cases estimated to be <0.0001% of the seed produced by the crop, i.e., less than one feral seed for 1,000,000 crop seeds (Messéan et al., 2009; Squire et al., 2011). This estimate for seed can also be taken as an absolute maximum for GM impurity arising through seed in the improbable event that all feral seed was harvested with the crop (Squire et al., 2011). So while several authors have cautioned that feral GMHT oilseed rape might be a significant concern in the management of coexistence of oilseed rape cropping systems, the recent quantitative evidence from demographic studies in Europe shows that its contribution to gene flow should be negligible compared to that from crop plants and volunteers. The only exceptions to this might be where occasionally very large populations of feral plants (e.g., > 10,000 plants) occur in derelict fields or around major construction works, adjacent to very small oilseed rape crop fields or oilseed rape certified seed production fields (SIGMEA, 2010; Squire et al., 2011), or in regions where a 'zero-tolerance' policy in terms of GM admixtures is in place (Demont and Devos, 2008; Devos et al., 2008; Ramessar et al., 2010; Sabalza et al., 2011).

7.2.2.2. Import scenario

Seed import spills of GMHT oilseed rape will be mostly confined to port areas. In the event that spillage, germination and flowering of a GMHT oilseed rape plant occurred in the ports and associated processing facilities, their location in industrial areas rather than agricultural areas makes it highly unlikely that gene transfer to the oilseed rape crop would occur (EFSA, 2004a). However, in the unlikely event that such gene transfer would occur, the concern may be that herbicide tolerance traits would enter agricultural fields and thus become cultivated unintentionally. Feral plants would in effect become volunteers, subject to the annual cycles of cropping and management. If the herbicides for which tolerance is obtained are applied as the sole agent of weed management in the field, then GMHT plants would not be controlled: herbicide tolerance traits could be amplified, subsequently causing a weed burden, and possibly requiring more stringent weed management. The introduced GMHT plants may set seed and replenish the soil seedbank. A worst-case scenario would be a persistence of the initial introduced GMHT oilseed rape plants. Therefore, the consequence might be: (1) the unintended cultivation of unapproved GM plants; (2) the subsequent gene flow to crop plants and stacking of herbicide tolerance traits; and (3) harvest admixtures. However, in the unlikely event that spilled seed would enter agricultural fields, the main opportunity of GMHT oilseed rape plants to reach maturity and produce seeds is one in every two to four years of the oilseed rape rotation, because standard herbicides used in oilseed rape do not control volunteer oilseed rape. Moreover, as no GMHT crops are currently approved for cultivation in the EU, the use of GLY and GLU is limited to two main timings in arable crops: pre-planting or pre-crop emergence to control a wide range of emerged weed species, and pre-harvest for late weed control or as a harvest desiccant to reduce moisture content (Cook et al., 2010). Therefore, exposure of the hypothesised in-field GMHT oilseed rape plants to GLY or GLU is expected to be limited.

7.2.2.3. Conclusion

Since feral plants derived from cultivation (as distinct from import) occur at too low a frequency to affect the tolerance threshold of 0.9% in the EU, even if they were assumed all to be transgenic, several authors concluded that feral GMHT plants resulting from seed import spills will have little

relevance as a potential source of pollen or seed for GM admixture in conventional oilseed rape crops (Messéan et al., 2009; Squire et al., 2011; Devos et al., 2012).

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that “*compared to cultivated oilseed rape populations, the contribution of feral oilseed rape plants in vertical gene flow is expected to be limited: feral oilseed rape populations are small compared to cultivated populations and contribute little to the pollen load in the environment (Colbach et al., 2001a,b, 2005; Devaux et al., 2005, 2007, 2008; Gruber and Claupein, 2006; Knispel et al., 2008; Colbach, 2009)*” (EFSA, 2009a).

The EFSA GMO Panel reiterates that issues pertaining to coexistence or GM admixtures are not in its remit (EFSA, 2006a, 2010).

8. GENE FLOW FROM FERAL OILSEED RAPE TO WILD RELATIVES

8.1. Concern raised by Austria

Austria raised the concern that feral oilseed rape Ms8 xRf3 plants will mediate transgene movement towards sexually compatible wild relatives.

8.2. Assessment by EFSA GMO Panel

8.2.1. Cultivation scenario

Oilseed rape is known to spontaneously hybridise with certain of its sexually compatible wild relatives (Scheffler and Dale, 1994; Devos et al., 2009a; Liu et al., 2010; Huangfu et al., 2011; Tsuda et al., 2011; de Jong and Hesse, 2012). Several oilseed rape x wild relative hybrids have been reported in the scientific literature, but under field conditions transgene introgression has only been confirmed for progeny of oilseed rape x *B. rapa* hybrids (Hansen et al., 2001, 2003; Warwick et al., 2003, 2008; Norris et al., 2004; Jørgensen, 2007). Due to ecological and genetic barriers, not all relatives of oilseed rape share the same potential for hybridisation and transgene introgression (Jenczewski et al., 2003; Chèvre et al., 2004; FitzJohn et al., 2007; Wilkinson and Ford, 2007; Devos et al., 2009a; Jørgensen et al., 2009). For transgene introgression to occur, both species must occur in their respective distribution range of viable pollen. This requires at least partial overlap in flowering in time and space, and sharing of common pollinators (if insect-pollinated) (Pascher et al., 1999, 2011; Wilkinson et al., 2000, 2003a; Chèvre et al., 2004; Simard and Légère, 2004; Allainguillaume et al., 2006; Simard et al., 2006; Wurbs et al., 2010). Sufficient level of genetic and structural relatedness between the genomes of both species also is needed to produce viable and fertile oilseed rape x wild relative hybrids that stably express the transgene (e.g., Heyn, 1977; Kerlan et al., 1993). Genes, subsequently, must be transmitted through successive backcross generations or selfing, so that the transgene becomes stabilised into the genome of the recipient. As no or only very low numbers of viable and fertile hybrids are obtained between oilseed rape and most of its wild relatives under ideal experimental conditions (e.g., through the use of artificial pollination and embryo rescue techniques in laboratory conditions (see FitzJohn et al., 2007)), Wilkinson et al. (2003b) concluded that exposure under real conditions is likely to be negligible, and the probability of transgene introgression is extremely small in most instances, with the exception of *B. rapa* in areas where it occurs close to oilseed rape (Vacher et al., 2011). Transgene introgression is likely to take place when oilseed rape and *B. rapa* grow in close proximity over successive growing seasons, especially if no significant fitness costs are imposed to backcross plants by transgene acquisition (Snow et al., 1999). However, hybrids between *B. napus* and *B. rapa* are mostly triploid with low male fertility, and hence low ability to pollinate and form backcrosses with *B. napus* (Norris et al., 2004). Incidences of hybrids and backcrosses with *B. rapa* were found to be low in fields in Denmark (Jørgensen et al., 2004) and UK (Norris et al., 2004). Recent observations in Canada confirmed the persistence of a GLY tolerance trait over a period of six years in a population of *B. rapa* in the absence of herbicide pressure (with the exception of possible exposure to GLY in one

year) and in spite of fitness costs associated with hybridisation (Warwick et al., 2008). A single GM *B. rapa* x *B. napus* hybrid was also reported along a road in Vancouver (Yoshimura et al., 2006), confirming the hybridisation possibility between these two *Brassica* species, albeit at very low frequencies. Elling et al. (2009) also described the detection of triploid hybrid offspring with intermediate morphology and oilseed rape microsatellite alleles from a single *B. rapa* mother plant. However, Elling et al. (2009) measured the extent of hybridisation between autotetraploid *B. rapa* varieties (female) and *B. napus* (pollen donor) under experimental field conditions, and found that hybridisation with tetraploid *B. rapa* seemed to be more likely than to diploid *B. rapa*. They reported that male fertility was higher in these hybrids than those formed with diploid *B. rapa* and suggested that introgression frequencies from *B. napus* to *B. rapa* would be higher in tetraploid *B. rapa*. They also reported the presence of some feral tetraploid *B. rapa* populations in northwest Germany, but did not report on interspecific hybrids or backcrosses in these populations.

8.2.2. Import scenario

Surveys and analyses conducted in Japan did not detect transgenes in seed collected from wild relatives (*B. rapa* and *B. juncea*) sampled at several ports and along roadsides and riverbanks (Saji et al., 2005; Aono et al., 2006). There have been very few other attempts to measure the transfer of genetic material from feral plants to wild relatives. Introgression of genetic material from feral oilseed rape to wild relatives, while theoretically possible, is likely to be very low due to the combined probabilities of spillage of GMHT oilseed rape in areas where wild relatives (e.g., *B. rapa*) are present, germination, survival of oilseed rape plants, hybridisation with its wild relatives, survival and the low fertility of interspecific hybrids restricting backcrossing with the wild relative.

8.2.3. Conclusion

Based on the available scientific literature, the EFSA GMO Panel concludes that introgression of genetic material from feral oilseed rape to wild relatives, while theoretically possible, is likely to be very low due to the combined low conditional probabilities of spillage of GMHT oilseed rape in areas where wild relatives (e.g., *B. rapa*) are present, of germination given spillage, of survival of oilseed rape plants given germination, of hybridisation with its wild relatives given survival, and of the survival and the low fertility of interspecific hybrids themselves, which restrict backcrossing with the wild relative.

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that “*feral oilseed rape populations may act as a genetic bridge delivering the (trans)genes to sympatric cross-compatible plants (Saji et al., 2005; Aono et al., 2006; Pascher et al., 2006; Yoshimura et al., 2006; Knispel et al., 2008; Nishizawa et al., 2009)*” (EFSA, 2009a).

The EFSA GMO Panel reiterates that it does not consider pollen dispersal and consequent hybridisation as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on biotic interactions and ecosystems by considering the spread and fitness of hybrids and backcross progeny (section 9; EFSA, 2006a, 2010).

9. IMPACT OF HERBICIDE TOLERANCE TRAITS ON FITNESS, PERSISTENCE AND INVASIVENESS OF FERAL OILSEED RAPE AND HYBRIDISING WILD RELATIVES

9.1. Concern raised by Austria

Austria raised the concern that herbicide traits may cause a change in fitness, leading to invasion of semi-natural habitats, and a colonisation of agricultural fields.

9.2. Assessment by EFSA GMO Panel

9.2.1. *Altered fitness, persistence and invasiveness*

The evidence on fitness, persistence and invasiveness of feral GMHT oilseed rape is derived from the following sources: (1) transplant or seed sowing experiments; (2) ecophysiological experiments and models on comparative fitness; and (3) demographic studies and surveys to see whether feral oilseed rape invades natural habitats (EFSA, 2010). Field studies in the first category have confirmed that herbicide tolerance traits in oilseed rape do not confer a fitness advantage, unless the herbicides for which tolerance is obtained are applied. In these studies, the invasive potential of GM plants was assessed directly by releasing them into natural habitats and by monitoring their fitness in subsequent generation(s). GMHT oilseed rape introduced into twelve different habitats at three sites across the UK failed to persist in established vegetation: in none of the natural plant communities considered was oilseed rape found after three years even when vegetation had been removed in the first year of sowing (Crawley et al., 1993, 2001). These experiments demonstrated that the genetic modification *per se* does not enhance ecological fitness (Hails and Morley, 2005; Hails et al., 1997).

Experiments and models on fitness differences between the GM plant and its non-GM counterpart (category 2 above) are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity (Fredshavn et al., 1995; Warwick et al., 1999, 2004, 2009; Norris and Sweet, 2002; Claessen et al., 2005a,b; Garnier and Lecomte, 2006; Garnier et al., 2006; Simard et al., 2005; Londo et al., 2010, 2011; Watrud et al., 2011). Beckie et al. (2004) showed that GMHT oilseed rape with single or multiple herbicide tolerance traits is not more persistent (weedier) than non-GMHT plants. Also greenhouse studies, in which the fitness of oilseed rape volunteers with no, single, or multiple HT was assessed, have shown no or little difference in fitness among oilseed rape plants in the absence of herbicide pressure (Simard et al., 2005). There is also no evidence that tolerance to GLY or GLU enhances seed dormancy, and thus the persistence of GMHT oilseed rape plants, compared to its conventional counterpart (Hails et al., 1997; Sweet et al., 2004; Lutman et al., 2005, 2008; Messéan et al., 2007). Seed dormancy (secondary dormancy, since there is little primary dormancy at seed shed), is more likely to be affected by the genetic background of parental genotypes than the acquisition of herbicide tolerance traits (López-Granados and Lutman, 1998; Lutman et al., 2003; Gulden et al., 2004a,b; Gruber et al., 2004; Messéan et al., 2007; Baker and Preston, 2008).

Demographic studies and surveys (category 3) have concluded that feral oilseed rape is confined to ruderal habitats and that feral GMHT oilseed rape does not behave as an ecologically hazardous invasive species (see Appendix B and references therein).

The evidence described above indicates that GMHT oilseed rape is neither more likely to survive, nor to be more persistent or invasive than its conventional counterpart in the absence of GLY or GLU. The ability of oilseed rape to successfully invade ruderal habitats appears to be limited principally by the availability of seed germination sites and interspecific plant competition, and there is no evidence that genes conferring HT significantly alter its competitive ability. Further, in controlled sowings into road verges, field margins and wasteland, very few seedlings survived to maturity due to grazing (e.g., by molluscs), plant competition and abiotic stress (Charters et al., 1999). Since GMHT oilseed rape has no altered survival, multiplication or dissemination characteristics, it is concluded that the likelihood of unintended environmental effects due to the establishment and spread of GMHT oilseed rape will be no different from that of conventional oilseed rape.

There is no evidence to suggest that herbicide tolerance traits in a wild relative changes its behaviour (Scheffler and Dale, 1994; Eastham and Sweet, 2002; Chèvre et al., 2004; Warwick et al., 2003, 2004, 2008; Jørgensen, 2007; Jørgensen et al., 2009), or the scale and nature of its interactions with associated flora and fauna (Wilkinson et al., 2003b; Wilkinson and Ford, 2007). Progeny from hybrids of oilseed rape and wild relatives that bear the herbicide tolerance trait do not show any enhanced

fitness, persistence and invasiveness, and behave as conventional counterparts, unless the herbicides for which tolerance is obtained are applied (Londo et al., 2010, 2011; Watrud et al., 2011).

GLU is used for general weed control in orchards and around field margins, banks and ditches, and could encourage increased persistence of GLU tolerant plants in these areas (section 10.2.1). In these areas, the GLU tolerance trait is likely to increase the fitness of GMHT plants (be it feral plants or progeny from hybrids of oilseed rape and wild relatives) relative to non-GLU tolerant plants when exposed to GLU (Londo et al., 2010, 2011; Watrud et al., 2011). As indicated previously, both the occurrence of feral GMHT oilseed rape resulting from seed import spills (section 4.2.2) and the introgression of genetic material from feral oilseed rape to wild relatives (section 8.2.3) are likely to be low under an import scenario. Therefore, feral oilseed rape plants and genes introgressed into other cross-compatible plants would not create significant agronomic or environmental impacts, even after exposure to GLU.

A trait that is expected to exert a negative effect on the fitness of feral GM oilseed rape is male sterility (i.e., the absence of pollen-producing anthers) which occurs in a proportion of seed produced by Ms8 x Rf3. Progeny may be male fertile or male sterile and have a variable number of copies of the *bar* gene, while a small proportion will have no *bar*, *barstar* or *barnase* genes. Male-sterile plants still produce stigmas and will set seed by pollen from another plant. They can therefore receive genes, but not transmit them by pollen. However, the effect of such male sterility on the fitness of feral individuals and populations has not been investigated in the field. The effect of such male sterility is to give high seed yields in selected oilseed rape varieties in fields, but is not likely to increase fitness of feral individuals and populations outside the field (Hails et al., 1997; Sweet et al., 2004; Lutman et al., 2005, 2008; Messéan et al., 2007).

9.2.2. Conclusion

Having reviewed all relevant scientific literature, the EFSA GMO Panel confirms that there is no evidence that the herbicide tolerance trait results in enhanced fitness, persistence or invasiveness of oilseed rape Ms8 x Rf3, its segregants, or hybridising wild relatives, unless they are exposed to GLU-containing herbicides. Escaped oilseed rape plants and genes introgressed into other cross-compatible plants would therefore not create significant agronomic or environmental impacts. Even in the worst case, considering data on gene flow, persistence and invasiveness derived from cultivation, where exposure and potential impact are expected to be the highest, the EFSA GMO Panel could not identify scientific evidence to indicate any significant and imminent risk to the environment arising from the authorised uses of oilseed rape Ms8, Rf3 and Ms8 x Rf3.

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that “*in the absence of glufosinate-ammonium-containing herbicide applications, oilseed rape MS8, RF3 and MS8xRF3 is neither more likely to survive, nor more invasive or persistent than its conventional counterpart. Only where and when glufosinate-ammonium-containing herbicides are applied, is oilseed rape MS8, RF3 and MS8xRF3 expected to have a fitness advantage. Likewise, there are no indications that the barstar/barnase gene complex would alter seed survival characteristics and confer a selective advantage (Fredshavn et al., 1995; Sweet et al., 2004; Lutman et al., 2008)*”. The EFSA GMO Panel also noted that “*there are no compelling data to suggest that the presence of an herbicide tolerance trait in a wild relative changes the behaviour of the wild relative so far (e.g., Warwick et al., 2008). In the absence of glufosinate-ammonium-containing herbicides, hybrids or wild relatives containing the herbicide tolerance trait do not show any enhanced fitness and behave as conventional plants*” (EFSA, 2009a).

10. MANAGEMENT

10.1. Concern raised by Austria

Austria raised the concern that feral oilseed rape Ms8 x Rf3 may cause or exacerbate herbicide management problems of roadside habitats due to the unintended stacking of herbicide tolerance traits.

10.2. Assessment by EFSA GMO Panel

10.2.1. Management

At present feral oilseed rape is not usually the specific target of road verge management in Europe, but in many areas roadside verges are mown or sprayed with herbicides as part of general control of vegetation by municipal or highway authorities (Charters et al., 1999; Knispel and McLachlan, 2009). A range of studies concluded that targeted control of roadside feral oilseed rape plants can be achieved mechanically (e.g., mowing) or chemically at a local scale (Beckie et al., 2004; Warwick et al., 2004; Simard et al., 2005; Gruber et al., 2008; Lutman et al., 2008), provided that monitoring systems are in place to detect where significant populations of feral oilseed rape exist (Beckie et al., 2010) and that any control measures taken are timely (Yoshimura et al., 2006).

GLU is used for general weed control in orchards and around field margins, banks and ditches, and could encourage increased persistence of GLU tolerant plants in these areas. In these areas, GLU tolerant plants (feral plants or progeny from hybrids of oilseed rape and wild relatives) are likely to have greater fitness relative to non-GLU tolerant plants when exposed to GLU (Londo et al., 2010, 2011; Watrud et al., 2011).

GMHT oilseed rape with single or multiple transgenes have been shown to remain controllable by the application of currently used herbicides with alternative modes of action (Beckie et al., 2004; Dietz-Pfeilstetter and Zwerger, 2009), or by mowing or cutting. Alternative herbicides (other than GLU) and mechanical removal or cutting are options for control of feral and volunteer plants. Repeated mowing of established plants during the season may be necessary to limit flowering and seed set by asynchronously developing populations (Garnier et al., 2006), but will similarly affect a broader range of non-target wild plant species. Since feral populations generally consist of a mixture of different (including spring- and winter-sown) varieties (Pascher et al., 2010), varying in morphology and phenology, with seedlings emerging and flowering at various rates and times in the season, management would need to be in tune with the feral life cycle (Crawley et al., 1993; Claessen et al., 2005a,b; Knispel and McLachlan, 2009).

Management efforts exclusively focused on controlling adult plants may not be sufficient to drive feral oilseed rape populations to local extinction in the short-term, and may even be counterproductive. The pattern and timing of mowing may vary, with varying effects on the reproductive success of feral plants. Further, ecological models predict that the regular mowing of vegetation and soil disturbance encourage the establishment of annual weed species including oilseed rape due to the creation of competition-free germination sites with reduced competition by perennial vegetation where new seed can establish and contribute to new feral plants (Claessen et al., 2005a,b; Garnier et al., 2006; Knispel, 2010; Bagavathiannan et al., 2012). Therefore, management efforts may also have to focus on limiting seed immigration from fresh seed spills (Knispel, 2010; Bagavathiannan et al., 2012; Bailleul et al., 2012). If total control of a population is warranted, repeated mowing and/or herbicide applications may be required until the exhaustion of the soil seedbank, as the presence of dormant seeds in the soil seedbank may contribute to new recruits over time (Bagavathiannan et al., 2012).

10.2.2. Conclusion

The EFSA GMO Panel does not regard the occasional occurrence of feral GMHT oilseed rape plants as an environmental hazard in itself. However, if the targeted control of roadside feral oilseed rape plants is considered desirable by risk managers, then this can be achieved chemically or mechanically (e.g., mowing) at a local scale, provided that monitoring systems are in place to detect where

significant populations of feral oilseed rape exist and that any control measures taken are timely. Risk managers can also implement appropriate communication means for the timely reporting of control failures of feral oilseed rape populations, as such observations may reveal the occurrence of feral GMHT oilseed rape plants, and may serve as a trigger for specific management. However, the EFSA GMO Panel draws the attention to evidence suggesting that management efforts that act to solely suppress adult survival may encourage the establishment of annual weed species including oilseed rape due to the creation of competition-free germination sites where new seed can establish and contribute to new feral plants. Therefore, management efforts exclusively focused on controlling adult plants may have to be complemented with measures limiting seed immigration from fresh seed spills. In addition, if total control of a population is warranted, repeated mowing and/or herbicide applications may be required until the exhaustion of the soil seedbank, as the presence of dormant seeds in the soil seedbank may contribute to new recruits over time.

This conclusion is consistent with that drawn by the EFSA GMO Panel in its Scientific Opinion on the safeguard clause invoked by Austria during 2008 on oilseed rape Ms8, Rf3 and Ms8 x Rf3 according to Article 23 of Directive 2001/18/EC (EFSA, 2009a). The EFSA GMO Panel considered that “*feral oilseed rape MS8xRF3 and hybridised/introgressed relatives can be managed by the use of other herbicides and/or adequate mechanical practices (Beckie et al., 2004; Devos et al., 2004; Warwick et al., 2004; Légère, 2005; Simard et al., 2005; Gruber et al., 2008; Lutman et al., 2008)*” (EFSA, 2009a).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel could not identify new scientific evidence in the documentation provided by Austria and in the scientific literature that indicated that the import and processing of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for feed uses in the EU pose a significant and imminent risk to the environment. The EFSA GMO Panel considers that, based on the documentation supplied by Austria, and a review of recent scientific literature, there is no specific scientific evidence in terms of risk to the environment that would support the notification of a safeguard clause measure under Article 23 of Directive 2001/18/EC nor its prolongation, and that would invalidate its previous risk assessments of oilseed rape Ms8, Rf3 and Ms8 x Rf3.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission, dated 22 May 2012, to the EFSA Executive Director requesting the assessment by EFSA of the scientific elements supporting the prolongation of prohibition of the placing on the market of GM oilseed rapes Ms8, Rf3 and Ms8 x Rf3 for feed purposes in Austria.
2. Acknowledgement letter, dated 11 July 2012, from the EFSA Executive Director to the European Commission.

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APPENDICES

A. OVERVIEW OF SURVEYS MONITORING TRANSGENE PRESENCE IN FERAL OILSEED RAPE POPULATIONS (ADAPTED FROM DEVOS ET AL., 2012)

Country	Surveyed area	Period	Transgene detection	Sampled material	Reference
Belgium	Roadsides nearby and field margins of cropped fields in Wallonia	2007-2008	DNA analysis	Leaf	Berben (2008, 2009)
	Port areas (Antwerp, Gent, Izegem and Kluisbergen)	Not specified	DNA analysis	Leaf	Mbongolo Mbella et al. (2010)
Canada*	Roadsides nearby and field margins of cropped fields in southern Manitoba (central Canada)	2004-2006	Herbicide screening & biochemical (protein) analysis	Seed, leaf	Knispel et al. (2008)
	Roadsides and railway lines in Saskatchewan and at the port of Vancouver	2005	Biochemical (protein) analysis	Leaf	Yoshimura et al. (2006)
Japan	Port areas (Kashima, Chiba and Yokohama), roadsides and riverbanks in the Kanto district	2004	Herbicide screening & biochemical (protein) analysis & DNA analysis	Seed	Saji et al. (2005)
	Port areas, roadsides and riverbanks in western Japan (Shimizu, Yokkaichi, Sakai-Senboku, Uno, Mizushima, Kita-Kyusyu and Hakata)	2005	Herbicide screening & biochemical (protein) analysis & DNA analysis	Seed	Aono et al. (2006)
	Port areas and roadsides in the area of Yokkaichi	2004-2007	Biochemical (protein) analysis	Leaf	Kawata et al. (2009)
	Roadside (Route 51) in eastern Japan	2005-2007	Biochemical (protein) analysis & DNA analysis	Leaf	Nishizawa et al. (2009)
Switzerland	Railway stations (31)	Not specified	Biochemical (protein) analysis & DNA analysis	Leaf	Schoenenberger and D'Andrea (2012)
USA*	Roadsides (interstate, state and country roads) in North Dakota	2010	Biochemical (protein) analysis	Leaf	Schafer et al. (2010, 2011)

* Country where GMHT oilseed rape is grown commercially

B. OVERVIEW OF DEMOGRAPHIC STUDIES OF FERAL OILSEED RAPE (ADAPTED FROM DEVOS ET AL., 2012)

Country	Surveyed area	Period	Proportion of oilseed rape in agricultural area	Reference
Austria	Roadsides, railway lines, fallow land, excavated soil and ruderal sites in Burgenland, Waldviertel and Innviertel	1998-1999	Moderate	Pascher et al. (2006, 2010)
Canada*	Roadsides nearby and field margins of cropped fields in southern Manitoba (central Canada)	2004-2006	High	Knispel and McLachlan (2009); Knispel (2010)
Denmark	Roadsides nearby and field margins of cropped fields in Mid-Jutland/Bjerringbro	2005-2006	Moderate	SIGMEA (2010); Squire et al. (2011)
France	Roadsides in Selommes (Loir-et-Cher)	1996-1997	High	Pessel et al. (2001)
	Roadsides nearby and field margins of cropped fields in Selommes (Loir-et-Cher)	2000-2005	High	Deville (2004); Pivard et al. (2008a,b); SIGMEA (2010); Squire et al. (2011)
Germany	Roadsides and field margins of cropped fields in northern Germany (Bremen)	2001-2003, 2005	Moderate	Menzel (2006); Reuter et al. (2008); SIGMEA (2010); Squire et al. (2011)
	Roadsides and field margins of cropped fields in northern Germany (Braunschweig)	2001-2004	Moderate	Dietz-Pfeilstetter et al. (2006); SIGMEA (2010); Squire et al. (2011)
	Roadsides and semi-natural habitats in northwest Germany (Lower Saxony)	2004-2007	Moderate	Elling et al. (2009)
Japan	Port areas and roadsides in the area of Kashima	2004-2005	Low	Mizuguti et al. (2011)
The Netherlands	Roadsides, railway lines and semi-natural habitats in oilseed rape cultivation areas, and the ports of Rotterdam and Amsterdam	2008-2009	Low	Luijten and de Jong (2010)
New Zealand	Road verges, drainage ditches, channels, natural watercourses, shelterbelts and wasteland in several plots in the region of Canterbury (South Island)	2003, 2005	High	Heenan et al. (2004); Peltzer et al. (2008)
United Kingdom	Roadside (M25) in southern England	1993-2002	Low	Crawley and Brown (1995, 2004)
	Roadsides nearby and field margins of cropped fields in the Tayside region (Scotland)	1993-1995, 2004	Moderate	Wilkinson et al. (1995); Charters et al. (1999); Bond et al. (2004); Banks et al. (2010); SIGMEA (2010); Squire et al. (2011)
	Field margins, hedges, roadsides and watercourses nearby cropped fields across the UK	1994-2000	Moderate	Norris and Sweet (2002)

* Country where GMHT oilseed rape is grown commercially